Association of platelet-activating factor acetylhydrolase gene polymorphism with premature coronary artery disease in Turkish patients

Türk hastalarda prematüre koroner arter hastalığı ile platelet-aktive edici faktör asetilhidrolaz gen polimorfizmi arasındaki ilişki

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ABSTRACT

Objective: Platelet-activating factor (PAF) is a phospholipid with multiple actions that is involved in inflammatory diseases as well as in atherogenesis. It is inactivated by a plasma enzyme, PAF-acetylhydrolase (PAF-AH). Deficiency of this enzyme in plasma is caused by a missense mutation in the gene (G994T). The aim of this study was to investigate association of this mutation with premature coronary artery disease (CAD).

Methods: One hundred and fifteen unrelated Turkish patients with a diagnosis of premature CAD and 128 unrelated healthy subjects were enrolled in this study. Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: The prevalence of the G994T mutation in the patients was 2.60 % (heterozygote), and 0 % in the controls. There was no significant difference in allele frequency and genotype distribution among the study groups.

Conclusion: The G9943T mutation in the plasma PAF acetylhydrolase gene is not associated with premature CAD in Turkish subjects. *(Anadolu Kardiyol Derg 2006; 6: 132-4)*

Key words: Premature coronary artery disease, PAF acetylhydrolase, gene, polymorphism

ÖZET

Amaç: Plazma platelet-aktive edici faktör (PAF), aterogenezis gibi inflamatuvar hastalıklarda rol oynayan multipl etkili bir fosfolipiddir. PAF, bir plazma enzimi olan PAF Asetilhidrolaz tarafından inaktive edilir. PAF-AH genindeki G994T mutasyonu, plazmada bu enzim düzeyinde azalmalara neden olur. Bu çalışmanın amacı prematüre koroner arter hastalığının (KAH) G994T mutasyonu ile ilişkisini araştırmaktır.

Yöntemler: Çalışmaya 115 prematüre KAH öyküsü olan ile 120 KAH öyküsü olmayan sağlıklı bireyler alındı. Her iki grubun genotip analizleri polimeraz zincir reaksiyonu (PZR) ve kısıtlayıcı parça uzunluk polimorfizm (RFLP) yöntemleri kullanılarak yapıldı.

Bulgular: G994T mutasyonu prevalansı hastalarda %2.60 heterozigot, kontrollerde ise % 0 olarak bulundu. Allel frekansı ve genotip dağılımı açısından hasta ve kontrollerde anlamlı bir fark olmadığı gözlendi.

Sonuç: Platelet-aktive edici faktör -AH geni G994T mutasyonu ile prematüre KAH arasında anlamlı bir ilişki olmadığı saptandı. *(Anadolu Kardiyol Derg 2006; 6: 132-4)*

Anahtar kelimeler: Prematüre koroner arter hastalığı, PAF asetilhidrolaz, gen, polimorfizm

Introduction

Platelet-activating factor (PAF) is a potent lipid mediator involved in inflammatory diseases as well as in atherogenesis (1). Platelet-activating factor is the common name for 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, identified in 1979 (2, 3). It is inactivated by the enzyme PAF acetylhydrolase, which removes the sn-2 acetyl group (4). This enzyme is widely distributed in mammalian tissues and blood, and also in contrast to other classical phospolipases A2 that are interfacial enzymes, does not cleave the sn-2 long chain fatty acids such as arachidonic acid. PAF-AH acts on its substrates in the aqueous phase and for this reason degrades essentially water soluble phospholipids (5) including PAF, in this case by hydrolyzing its acetate moiety (2 carbons) in the sn-2 position of glycerol (4).

The gene that encodes the enzyme PAF-AH is located on chromosome 6q21.2-p12, and it consists of 12 exons (6). Stafforini et al. identified a missense mutation in the gene of plasma PAF acetylhydrolase (G994T, Val279Phe) as the cause of deficiency of enzyme activity (7). They showed that this mutation as a heterozygous trait is 27% in the Japanese population.

Yamada et al. (8) reported that the V279F mutant allele was significantly higher in Japanese male patients with myocardial infarction (heterozygous, 33.0%; homozygous, 2.1%) than in controls (heterozygous, 21.0%; homozygous, 2.2%). By contrast, the

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V279F mutant allele was not associated with myocardial infarction in women. Hiramoto et al. (9) demonstrated that the prevalence of the V279F mutation was significantly higher in Japanese patients with stroke (heterozygous, 39.2%; homozygous, 4.2%) than in controls (heterozygous, 22.4%; homozygous, 3.0%). These results suggest that the V279F mutation of plasma PAF-AH may be a genetic risk factor for atherosclerotic diseases.

It has been reported that the mutation is a risk factor for myocardial infarction in men, stroke, atherosclerotic occlusive disease and abdominal aortic aneurysm in Japanese population (7-11). In addition, this mutation is associated with non-familial dilated cardiomyopathy (12), nonfamilial hypertrophic cardiomyopathy (13) and cerebral hemorrhage (14) in Japanese patients.

The aim of this study was to investigate association of a missense mutation in plasma PAF acetylhydrolase (G994T) with premature coronary artery disease (CAD).

Materials and Methods

Platelet-activating factor acetylhydrolase gene polymorphism was analysed in 115 unrelated Turkish patients with a diagnosis of premature CAD who were admitted to the Cardiology Department of the three centers in the Eagean region, West of Turkey. The Control group consists of 128 unrelated healthy subjects without a history of CAD. The study was approved by the Ethics Committee of the Celal Bayar university hospital, and all subjects provided written informed consent. The inclusion criteria for the patients were: 1) age at the time of CAD diagnosis 55 years or less in men and 65 years or less in women; 2) stenosis of at least 50% in a major coronary artery, or one of their branches, as determined by angiography. The extent of disease was defined as the number of arteries with stenosis at least 50% as single or multiple vessels. The coronary angiography was performed by Judkin's method at the Catheterization Laboratories. Diagnosis of myocardial infarction (MI) was ascertained from patients records using the WHO criteria (15) based on symptoms, elevation in cardiac enzymes or electrocardiographic changes.

All patients provided information about coronary risk factors such as diabetes mellitus, hypertension, hypercholesterolemia and cigarette smoking. Triglycerides, total cholesterol, high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL) levels were measured by conventional methods of clinical chemistry. Arterial hypertension was defined as systolic blood pressure equal to or greater than 140 mmHg and/or diastolic blood pressure equal to or greater than 90 mmHg in more than one determination. Patients with a history of diabetes or basal glucaemia greater than 120 mg/dl were defined as diabetic. Smoking habit was defined as a daily intake of more than 5 cigarettes. Body mass index was defined as increased when greater than 25 kg/m2. A family history of CAD was determined by interviewing patients and controls.

Genomic DNA was isolated from peripheral blood by standard methods. Exon 9 of the PAF acetylhydrolase gene was amplified by using the primers described before (7). The 177-bp PCR products were digested with TaiI at 56°C overnight. Digestion products were subjected to electrophoresis on 8% polyacrylamide gel and stained by ethidium bromide. The 95- and 82-bp DNA fragments indicated the presence of the mutation (16).

Statistical analysis

Statistical analysis was carried out with SPSS program for Windows 98 version 10.0 (SPSS Inc., Chicago, IL, USA). Variables are presented as means ± SD. P value of 0.05 or less was considered as significant. Univariate analysis was performed by Chi-square, odds ratios (OR) and Mann Whitney U test.

Results

The study population consisted of 115 patients with premature CAD and 128 control subjects. The clinical characteristics of the patients and controls are summarized in Table 1.

The patients (73.0 %) and controls (74.2 %) were predominantly men. The patients group had a higher prevalence of hypertension (40.9 %), diabetes (21.7 %), smoking (63.5 %) and family history of premature CAD (40.8 %) compared with the controls.

The prevalence of G994T mutation was found heterozygous 2.60% in patients with premature CAD and 0 % in controls. The allele frequencies and the genotype distributions were not significantly different between patients and controls (Table 2).

Discussion

In this study, it has been shown that, the G994T mutation in the plasma PAF acetylhydrolase gene is not associated with premature CAD in Turkish subjects.

Miwa et al. first reported that the deficiency of plasma PAF-AH activity, one of the factors playing a role in the pathogenesis of CAD, was transmitted by autosomal recessive heredity in five Japanese families (17). Most instances are due to a loss of function mutation (Val279Phe, exon 9, position 994; G´T) in the plasma PAF-AH gene (7). The Val-279 position in plasma PAF-AH is conserved from different species, and this amino acid lies between the active site Ser-273 and Asp-296 residues in a region that is critical for proper folding of the enzyme. These results suggested that the change of valine (Val) to phenilalanine (Phe) in codon 279 may cause to defect in plasma PAF-AH activity. The incidence of the mutation is reported to be high in healthy Japanese (heterozygous, 27 %; homozygous, 4 %). It was suggested that the V279F mutation of plasma PAF-AH AH may be a genetic risk factor for atherosclerotic diseases. In addition, this mutation is associated with nonfamilial hypertrophic cardiomyopathy, non-familial dilated cardiomyopathy, cerebral hemorrhage, and renal failure in Japanese patients (12-14, 18, 19).

A study by Balta et al., first reported the existence of the mutation in non-Japanese populations (16). In that study, 358 unrelated healthy Turkish, 143 Kyrgyz and 100 Azeri people were investigated and among these subjects heterozygous mutations were

Table 1. The demographic characteristics and distribution of risk factors in patients and controls

	Patients(n=115)	Controls (n=128)
Age, years	47.1 ± 5.7	46.2 ± 6.1
Male/female, n (%)	84/31(73.0/ 27.0)	95/33(74.2/25.8)
BMI, kg/m2	26.6 ± 2.3	24.7 ± 2.8
Diabetes, n (%)	25 (21.7)	4 (3.1)
Family history of CAD, n (%)	46 (40.8)	11 (8.6)
Hypertension, n (%)	47 (40.9)	18 (14.1)
Smoking habit (≥5/day) , n (%)	73 (63.5)	42 (32.8)
Total cholesterol, mg/dl	204.8 ± 33.2	179.6 ± 23.7
HDL cholesterol, mg/dl	40.8 ± 3.3	44.1 ± 4.2
LDL cholesterol, mg/dl	130.6 ± 25.1	122.1 ± 26.3
Triglycerides, mg/dl	181.4 ± 74.6	155.6 ± 51.2
Single vessel disease, n (%)	46 (40.8)	-
Multiple vessel disease, n (%)	69 (59.2)	-

BMI- body mass index, CAD- coronary artery disease, HDL- high density lipoprotein cholesterol, LDL- low density lipoprotein cholesterol

found in 3 (0.84 %), 12 (8.4 %) and 0 subjects, respectively. In our study, it was demonstrated that 3 patients had the mutation in heterozygous state (2.60 %), like in Balta et al.'s study (Table 3).

There are several limitations in this study. The first limitation concerns the sample size of the study. We examined the distribution of the V279F mutation of plasma PAF-AH in 142 control subjects and 164 patients, which is not a large sample. Secondly, there was a relatively small number of patients with the PAF-AH T allele. The small sample limited the statistic power of our study, determining a wide confidence interval, but the prevalence of the PAF-AH T allele in healthy Turkish people is very low [3 (0.84 %) and 12 (8.4 %) in 358 unrelated healthy Turkish and 143 Kyrgyz subjects, respectively]. Therefore, a larger sample should be examined to confirm the relation between this polymorphism and premature CAD, or existence of this mutation in Turkish population.

In conclusion, it was found that there was no significant relationship between the Val279Phe mutation in the gene of plasma PAF acetylhydrolase and premature CAD. Also, it has been shown that this mutation exists in Turkish population rather than in Japanese.

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Table 2. The genotype and allele frequencies of Val279Phe mutation in the PAF-AH gene

	Patients(n=115)	Controls (n=128)
PAF genotypes		
GG, n (%)	112 (97.40)	128 (100)
GT, n (%)	3 (2.60)	-
TT, n (%)	-	-
Allele frequencies G/T, n	0.870 / 0.130	1.0 / 0.0
PAF- platelet-activating factor		

Table 3. Prevalence of PAF-AH Val279Phe mutation in some populations studied

	Total number of subjects, n	Heterozygote, n (%)	Homozygote, n (%)	Total number of chromosomes, n	Positive mutation, n	Allele frequency, %
Turkish[1]	115*	3 (2.60)	-	230	3	1.3
Turkish[1]	128	-	-	256	-	-
Turkish[2]	358	3 (0.84)	-	716	3	0.42
Turkish-Azeri[2]	100	-	-	200	-	-
Turkish Kyrgyz[2]	143	12 (8.4)	-	286	12	4.2
Japanese[3]	270	74 (27.4)	5 (1.9)	540	84	15.6

[1]Present study
[2]Reference 17 data
[3]Reference 15 data
*patient subjects, others are healthy subjects.
PAF- platelet-activating factor